SEM studies on immature stages of *Pheidole indica* Mayr, 1879 (Hymenoptera: Formicidae) from India

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Abstract

The present work gains significance in the light of use of certain larval characters to species level taxonomy and systematics in various ant genera. Due to widespread distribution of some ant taxa it becomes mandatory to elucidate any interspecific and geographic variations. Keeping in view, the significance of immature stages in ant taxonomy, SEM studies on immature stages of *Phediole indica* Mayr, has been carried out to provide an insight into minute morphological details.

Keywords: *SEM, Immature stages, Pheidole indica, India.*

Introduction

With more than 12,000 species known, ants have emerged as a major insect group in terms of their significance in ecology, biology, natural history and evolution. With such diversity, immature stages of only few ant species have been described. The significant contributions have been by Wheeler and Wheeler (1976, 1986), who synthesized their previous works on immature stages covering about more than 700 ant species. Thus they paved the way for usage of larval characters in discriminating allied species. But unfortunately their work was not based or targeted at a particular species or species complexes; rather they provided a holistic view of immature morphology of various subfamilies of ants. Recent advances have been by Fox *et.al.* (2007) who carried SEM on larvae of *Paratraechina longicornis* and provided significant characterization of immatures.

As evident from previous works, the larvae of myrmicinae are so heterogenous that no characterization can be given for the subfamily as a whole. Genus *Pheidole* with more than 1,000 species worldwide considered hyper diverse has been poorly studied in terms of immature stages. However, some contributions have been made by Wheeler and Wheeler on subfamily Myrmicinae (1960a and b, 1980, 1989a,b and c, and 1991). As far as genus *Pheidole* is concerned Diana Wheeler (Personal communication) and Passera (1974) has worked on *Pheidole vinelandica* and *Pheidole pallidula*.

Keeping in view, the significance of immature stages in ant taxonomy, SEM studies on immature stages of *Pheidole indica* Mayr (distributed widely in countries like India, Japan and China) have been carried to provide an insight into minute morphological details, which could be used in future interspecific diagnosis.

Materials and Methods

The colonies of *Pheidole indica* Mayr were collected from Shivalik range of North-West Himalaya. The immatures were fixed in Dietrich's solution for 24-48 hrs and preserved in 80% alcohol. The larvae were separated into three instars according to their maximum head capsule widths. Measurements were made with compound microscope equipped with an ocular micrometer. After separation, larvae (N=10) from each instar were prepared for scanning electronic microscopy analysis with following steps;

a) Samples were post-fixed in 1% osmium tetraoxide, after which they were dehydrated in a graded acetone series.

b) Then the specimens were vacuum dried in desiccator.

c) Specimens adhered to the double-face-adhesive carbon tape were coated with gold in a gold ion sputter coater (HITACHI-E-1010).

d) Samples were analyzed under a HITACHI-S-3400N Scanning microscope.

For larval description, the terminology given in Wheeler and Wheeler (1976) and Fox *et al.* (2007) was followed.

Body hairs were measured at full length and body length as straight length. Measurements of the head capsule, mouthparts, hairs etc. were taken of one individual per instar on which the descriptions were based.

Results and Discussion

During present study based on SEM, three larval instars have been recognized in case of *Pheidole indica,* which are discussed here in detail.

First larval instar

Body-Whitish, Pheidoloid in shape due to the presence of short, stout and straight abdomen. Head ventral near anterior end, mounted on short stout neck, which is the prothorax (Fig. 1a). Hairs present on the entire body surface, but more abundant on the dorsal side than on the ventral side. Two types of hairs present on the body, anchor tipped hairs, measuring about 159 µm in length present mostly on the dorsal side of the body; and deeply bifid hairs, tips curling in opposite directions measuring about 49.07 µm in length; (Fig. 1c, 1d). Body length-1310µm (Fig. 1b).

Head capsule- Cranium 205 µm high x 222 µm wide; roughly sub-circular (Fig. 1e). Head surface smooth, two types of hairs present on the head which were distributed symmetrically; slightly curved or straight hairs, about 37.6 µm long; deeply bifid hairs about 40.1 µm long (Fig. 1e, 1f, 1g).

Mouthparts-Clypeus clearly delimited from the cranium, about 76.7 µm long, upper surface of clypeus smooth with two types of hairs; simple hairs about 27.1 µm long and tip bifid hairs about 26.2µm long (Fig. 1e, 1h). Labrum bilobed, 59.9 µm wide (Fig. 1g, 1h). Mandibles subtriangular pheidoloid in shape; with three subapical teeth, not all in the same plane; apex curved medially to form a tooth, about 87.7 µm long x 18.5 µm wide (Fig. 1g). The rounded fleshy maxillae protrude ventrolaterally from each side of the head. Maxilla paraboloidal in shape. The only sharply defined parts of the maxilla are the palp and galea, which are on the stipes. Galea about 23.8 µm long is longer than maxillary palp about 20.6 µm long x 13.6 um wide (Fig. 1i).

Second larval instar

Body- Change in the body shape has been observed in the second instar i.e shape found to be Attoid (stout and more curved; diameter approximately equal to the distance from labium to anus (Fig. 2a, 2b); anus clearly subterminal in position with lower lip bigger than the upper lip about 116 µm in diameter (Fig. 2a, 2c, 2d). Body hairs denser over the dorsal side of the body and of one type only i.e. bifid hairs about 58.55 µm in length (Fig. 2b, 2e). Body length-2100 µm (Fig. 2b).

Head capsule- Cranium 232 µm high x 262 µm wide. Hairs on the cranium are of two types, a distinct row of 4-5 simple slightly curved or straight hairs line the distal cranial border, and hairs bifid at the tip, about 43.7 µm long (Fig. 2f).

Mouthparts- Clypeus clearly delimited from the cranium, 79.3 µm long (Fig. 2f). Labrum bilobed 76.4 µm wide. Mandibles Pheidoloid in shape with two visible teeth, and measure about 81.3 µm long x 25.2 µm wide. Protuberances on the mandibles clearly visible (Fig. 2g). Maxilla paraboloidal in shape. Galea 24.1 µm long x 12.9 µm wide. Maxillary palpus 25.7 µm long x 16.1 µm wide (Fig. 2g).

Third larval instar

Body- Body profile same as in the second larval instar i.e attoid (Fig. 3a). Anus subterminal in position about 268 µm in diameter (Fig. 3b). Body hairs of three types; long bifid hairs about 47.17 µm long on the dorsolateral side; short bifid hairs about 13.3 µm long on the ventral side; tip denticulate hairs about 133 µm long (Fig. 3c, 3d). Body length-3020 µm.

Head capsule- Cranium 312 µm high x 236 µm wide (Fig. 3e). Hairs on the head are of two types (1) simple, slightly curved or straight hairs about 42.7 µm long; (2) bifid hairs about 27.75 µm long (Fig. 3e).

Mouthparts- Clypeus 84.3 µm long clearly delimited from the cranium; A distinct row of two simple slightly curved or straight hairs about 16.8 µm long lining the distal clypeal border (Fig. 3f, 3g). Labrum markedly bilobed 98.8 µm wide (Fig. 3f). Mandibles 70.5 µm long (Fig. 3h). Maxillae paraboloidal in shape. Galea 29 µm long x 10.9 µm wide. Maxillary palp 28.1 µm long x 14.7 µm wide (Fig. 3h).

The present study confirms the general aspects originally observed by Wheeler and Wheeler (1976, 1986) in case of genus *Pheidole* but some significant differences have been observed. First, variation in the type of hair especially over the head capsule between different larval stages has been observed (Fig. 1d, 1e, 2f, 3e). This variation was not noticed by Wheeler and Wheeler (1976), because their work was based on a single mature worker larva only. Hair type is one of the characters considered in calculating the "specialization indices" proposed by Wheeler and Wheeler (1986). Furthermore, the presence of bifurcations in the head hairs has been recently proposed as a character of considerable importance for separating species in the genus *Solenopsis* Westwood (Pitts *et al.*, 2005).

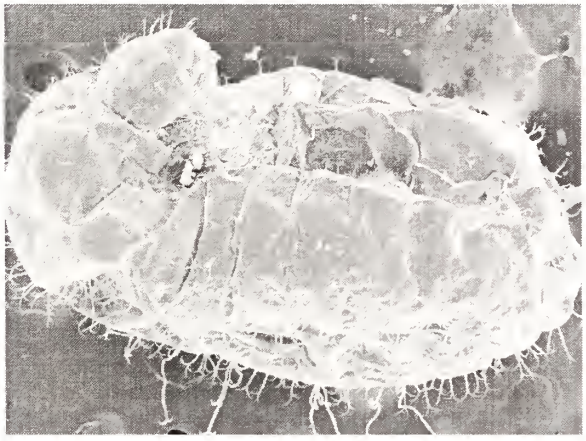


Fig.1a

Fig.1b

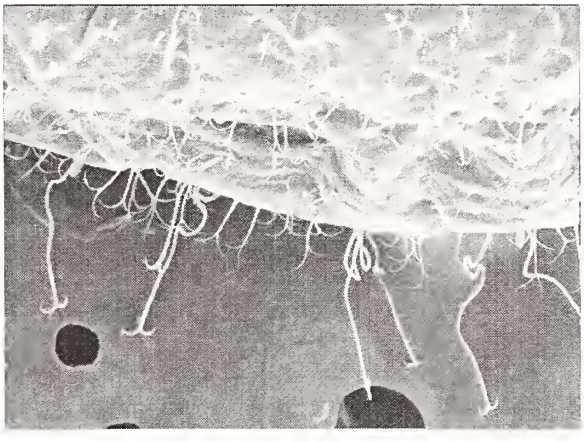


Fig.1c

Fig.1d

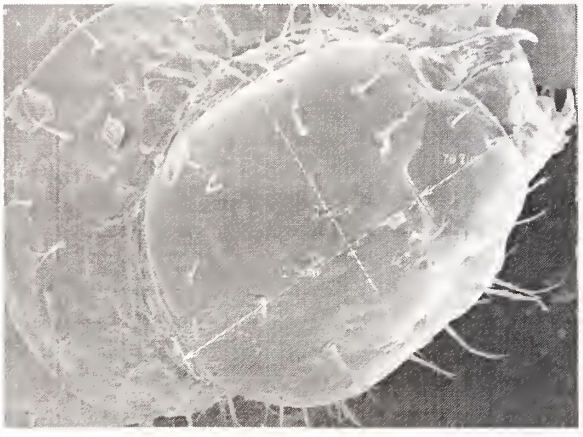


Fig.1e

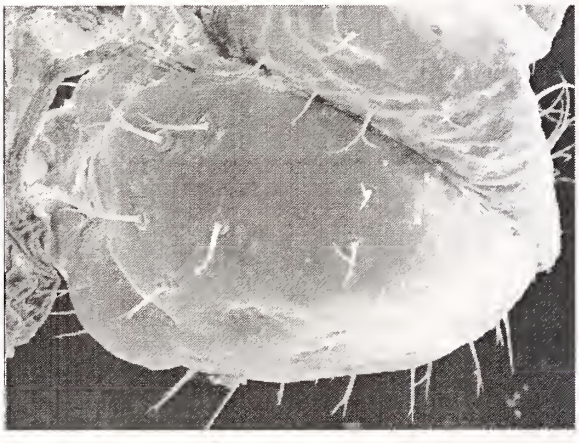


Fig.1f

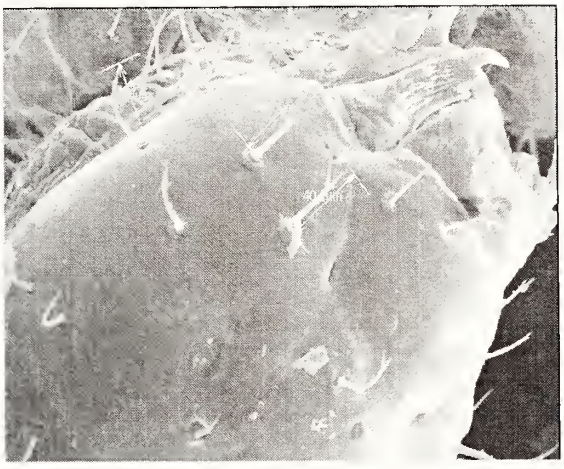


Fig.1g

Fig.2a

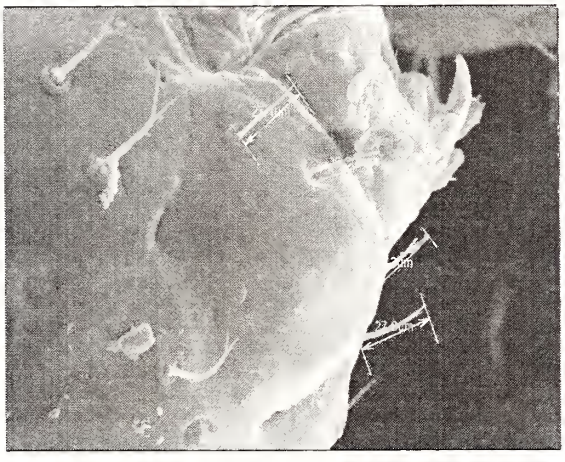


Fig.1h

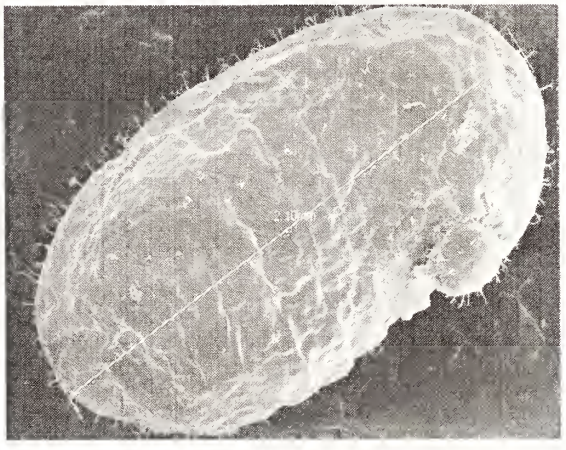


Fig.2b

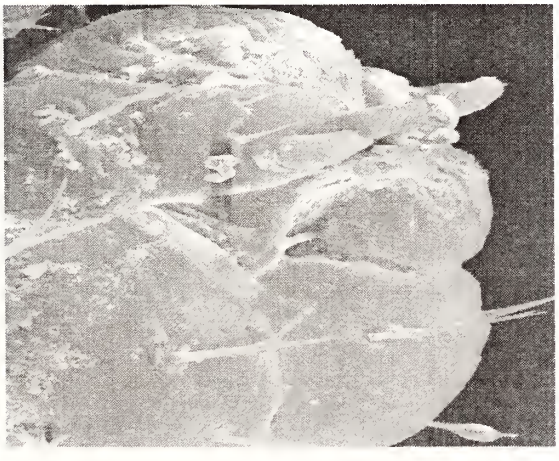


Fig.1i

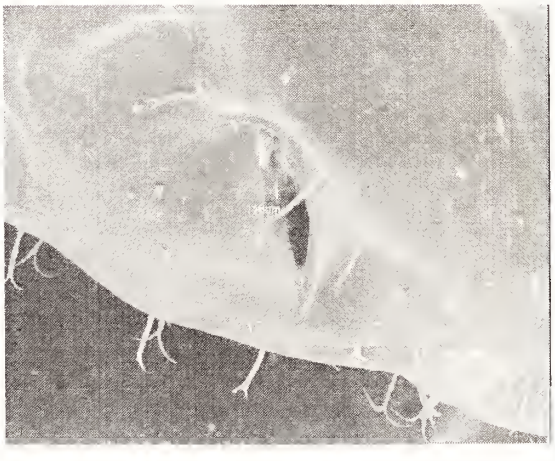
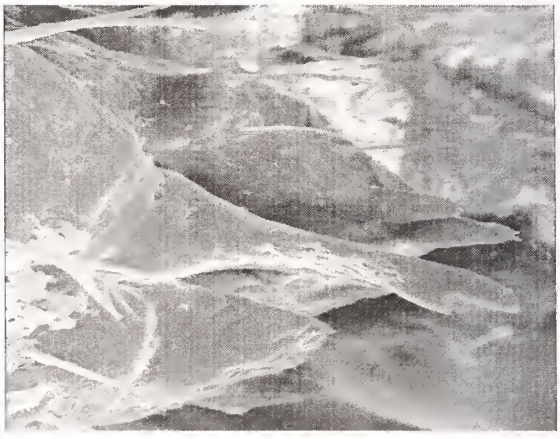


Fig.2c

Fig.2d



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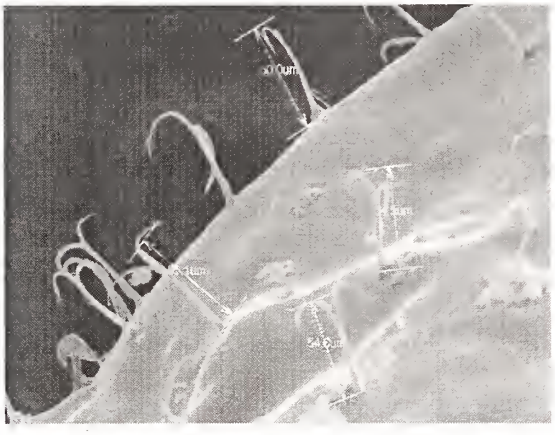


Fig.2e

Fig.3a

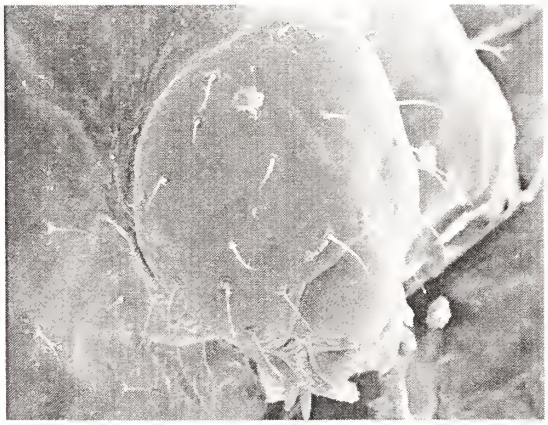


Fig.2f

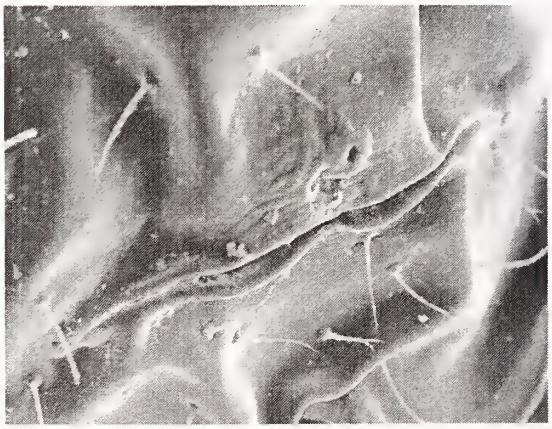


Fig.3b

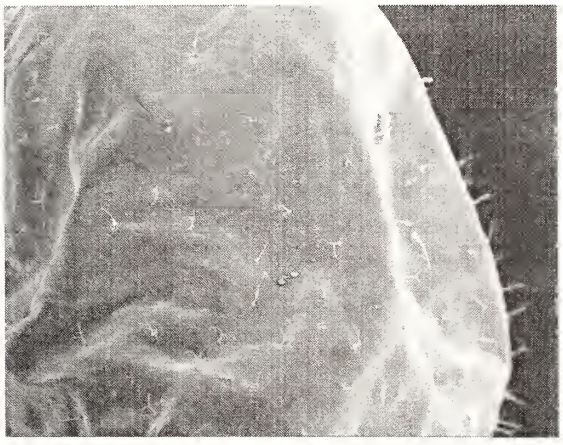


Fig.3c

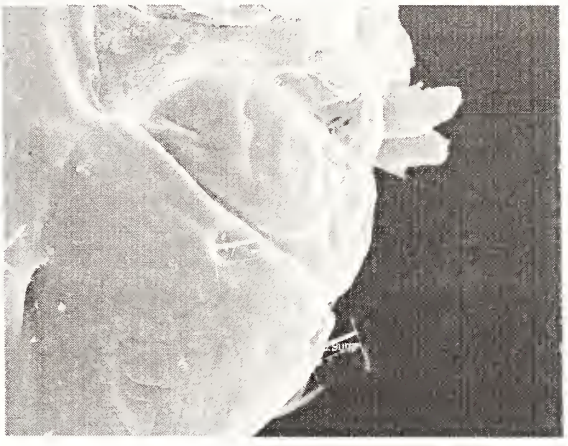


Fig.3f

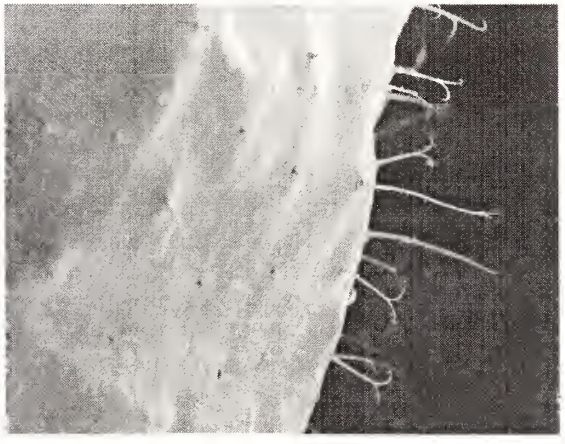


Fig.3d

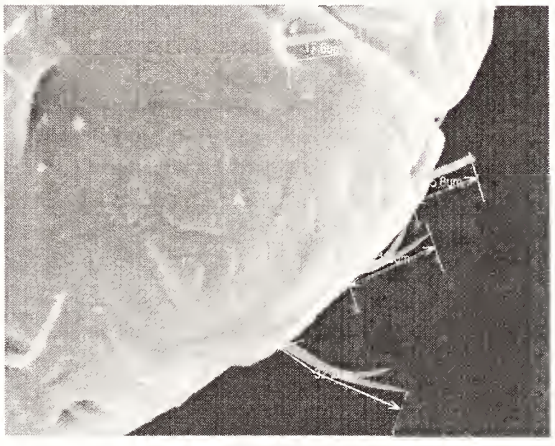
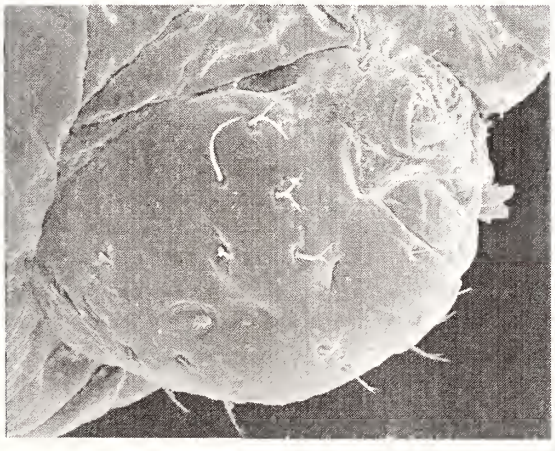


Fig.3g



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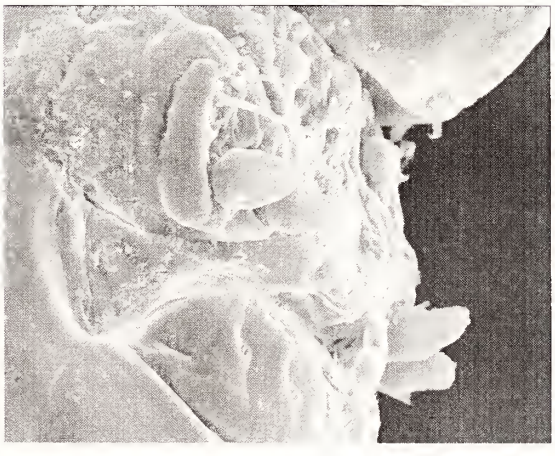

Fig.3h

Fox *et al.* (2007) pointed out that intraspecific variation in types of head hairs might occur in other ant species as well. Therefore, Fox *et al.* (2007) and the results of the present study clearly suggest the revision of previously described ant larvae in the light of present work. Two types of hairs has been observed on the cranium. Clear variation in the head capsule width between the three instars has been observed. It is noticed that the head capsule width considerably increases as the larvae matures. Clear variation in the distribution and as well as type of body hairs has been observed in the three instars. Two types of body hairs have been observed (anchor tipped hairs and deeply bifid hairs) in case of first instar. Anchor tipped hairs have been observed only on the dorsal surface of first instar (Fig. 1b, 1c). In the second larval instar only one type of hair are present i.e deeply bifid hairs (Fig 2e). In case of the third larval instar three types of hairs are present; long bifid hairs, short bifid hairs and tip denticulate hairs (Fig. 3c, 3d).

A clear variation in the body shape has been observed among the three larval instars. First, larva was found to be of pheidoloid in shape (Fig. 1a) as compared to the attoid type of body shape found in second and third larval instar (Fig. 2a, 2b and 3a). Body length of first, second and third instars is found to be 1310 µm, 2100 µm, 3020 µm respectively (Fig. 1b, 2b and 3a). Position of anus was sub-terminal in case of second and third larval instar with diameter 116 µm and 268 µm respectively (Fig. 2c and 3b).

Size of the cranium of first, second and third instars was 205 µm high x 222 µm wide, 232 µm high x 262 µm wide and 312 µm high x 236 µm wide respectively (Fig. 1e, 2f and 3e). Clypeus is found to be clearly delimited from the cranium in all the three instars and is 76.7 µm long, 79.3 µm long and 84.3 µm long in first, second and third instars respectively (Fig. 1e, 2f and 3e).

Labrum has been markedly bilobed and is 59.9 µm wide in first instar, 76.4 µm wide in second instar and 98.8 µm wide in third instar respectively (Fig. 1g, 2f and 3e). Mandibles subtriangular, pheidoloid in shape with two to three subapical teeth, apex curved medially to form a tooth (87.7 µm long x 18.5 µm wide) in case of first instar (Fig. 1e), whereas in case of second instar the number reduces to two and in third instar single apical tooth has been present (Fig. 2f).

Maxillae found to be paraboloidal in shape in all the three instars. Maxillary palps and galeae of all the three instars have been found to be very distinct. (Fig. 1i, 2g and 3h).

To conclude, further details to the description of the larvae of *Pheidole indica* have been added, which would help in future inferences in ant systematics and phylogeny.

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